MEMORANDUM

Date: December 8, 2008

From: Marie Csete, MD, Ph.D., Chief Scientific Officer

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application RT1-01064-1

Enclosed is a letter from Dr. Bruce Dunn, of University of California Los Angeles, an applicant for funding under RFA 08-02, CIRM Tools and Technologies Awards. This letter was not received at CIRM at least five working days prior to the December ICOC meeting, but we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

Since the letter was submitted late, scientific staff has not reviewed the petition.

The enclosed letter represents the views of its author(s). CIRM assumes no responsibility for its accuracy.

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December 5, 2008

Dr. Alan Trounson
President
California Institute for Regenerative Medicine

Dr. Marie Csete
Chief Scientific Officer
California Institute for Regenerative Medicine

RE: RT1-01064-1: Nano/Micro Technology Platform for the Promotion of Lineage Specific Differentiation of Pluripotent Stem Cells

Dear Dr. Trounson and Dr. Csete,

This letter concerns the content of the scientific review of the above proposal. There are three items which I would like to bring to your attention:

1. We were criticized for not comparing our methods with papers in the literature. One of the publications identified by the reviewers (Zandstra – Stem Cells 2008) was published in September, 2008, well after the proposal submission date. It is not realistic to have expected us to include that paper in our review.

2. Our manuscript (reference #21 in the proposal) has now been published. This manuscript was in-review at the time we submitted the proposal,. The citation is:

> B. Valamehr, S. J. Jonas, J. Polleux, S. Guo, K. Kam, R. Qiao, B. Stiles, E. H. Gschweng, T-J M. Luo, O. N. Witte, X. Liu, B. Dunn, H. Wu, "Hydrophobic Surfaces for Enhanced Differentiation of Embryonic Stem Cells Derived Embryoid Bodies", *PNAS* **105**, 14459-14464 (2008)

The careful, interdisciplinary research described in this paper is a strong indication of the commitment that my colleagues and I have to the field. It also underscores the importance of assembling a strong interdisciplinary team that we believe is vital for future studies.

3. Our grant fills a unique niche of integrating microfluidics, patterning and materials approaches to promote homogeneous and controlled differentiation into specific lineages. While there are a few recommended proposals that involve 'microfluidics', these proposals are directed towards very different objectives.

Thank you for your consideration.

Sincerely,

Bruce Dunn

Bruce Dunn
Nippon Sheet Glass Professor
Materials Science and Engineering

cc: O. N. Witte, M.D.

RT1-01064-1: Nano/Micro Technology Platform for the Promotion of Lineage Specific Differentiation of Pluripotent Stem Cells

Recommendation: Recommended if funds available
Scientific Score: 70
First Year Funds Requested: $452,782 **Total Funds Requested:** $905,564

Public Abstract (provided by applicant)

Embryonic stem cells (ESCs) and induced pluripotent stem (iPS) cells have generated tremendous interest in the scientific community because of their unique ability to differentiate or transform themselves into any of the specialized cell types found in the human body. These cells may therefore represent a source material for organ and tissue replacement to treat several debilitating ailments ranging from diabetes to Parkinson's disease. Unfortunately, the current methods for directing stem cell differentiation are notoriously inefficient and difficult to control. Scientists currently lack the tools to properly obtain the large populations of differentiated cells needed for these future clinical applications. For example, traditional culture practices and tools result in unsynchronized growth and differentiation of stem cells which have been shown to reduce yields of desired cell types. The work proposed in this project will address this problem by adapting engineering tools and methodologies which have been applied, over the past decade, to areas ranging from the automotive industry to microelectronics. Functionally, our proposed tools revolve around a common theme where we rigorously control the size of the initial stem cell colony or, in other strategies, aggregates known as embryoid bodies (EBs) prior to differentiation. One objective of our research is to assess the extent to which size control influences the outcome of lineage specific differentiation and then determine an optimal size range. A second objective is to develop engineering tools that regulate the addition of molecules called induction factors to the culture media that bathes the stem cells. Exposure to combinations of these signaling molecules guides the stem cells toward a preferred lineage. Using microfluidics, we will be able to exquisitely control the concentration and combination of these differentiation inducers and determine whether we can further increase differentiation yields. Altogether, these tools represent an enabling set of technologies that will permit scientists to more closely probe the mechanisms behind stem cell differentiation. This research provides the fundamental basis for a technology that has the potential to produce large populations of differentiated cells that will speed the translation of stem cell technologies for therapeutic and diagnostic applications.

Statement of Benefit to California (provided by applicant)

There has been tremendous interest in harnessing the unique capability of pluripotent stem cells to both self renew and to differentiate into all specialized cell types found in the human body. The scientific investigation of cells with these capabilities, such as human embryonic stem cells (hESCs) or induced pluripotent stem (iPS) cells, may eventually lead to revolutionary cell based treatments against several debilitating pathologies that affect many citizens of California such as type I diabetes mellitus, Parkinson's disease, and sickle cell anemia. While there is significant promise for this work, the transition from the laboratory bench toward clinically viable therapies has been slowed by several limitations. In particular, current state-of-the-art methodologies for directing stem cell differentiation toward specific lineages are notoriously inefficient and often result in mixed populations of cells. In order for the state of California to maintain its scientific leadership position in the emerging field of stem cell biology and ultimately drive the deployment of clinically relevant stem cell therapies to its citizens and the world, there is a critical need for new tools and strategies that will improve the efficiency and scalability of stem cell differentiation. The work proposed in this project integrates sophisticated engineering tools of microelectromechanical systems, microfluidics and synthetic materials into devices and systems that will move towards improved differentiation yields. The hypothesis-driven studies described in this proposal enable us to emphasize experimental parameters which are not attainable by traditional culturing practices as the engineering tools provide rigorous control over the microenvironment surrounding the differentiating stem cells. The engineering tools developed in this program form the basis for a technology which is scalable and robust, which will not only benefit the scientific community but will also drive emerging commercial and medical development of stem cell therapeutics and other advances that will the benefit the state of California and its citizens.

Review

In order to promote homogeneous and controlled differentiation of human embryonic stem cells (hESC) into specific lineages, the applicant proposes to develop new technology tools to improve control over various cell culture parameters. The approach employs two basic in vitro differentiation strategies, the formation of embryoid bodies (EBs) and differentiation in monolayers. Since EB size heterogeneity is a major source of noise when differentiating hESCs, the applicant proposes to achieve a narrow EB size distribution by applying microfluidics and microelectromechanical systems technologies to sort EBs, exert control over initial colony size, and to deliver differentiation inducing factors in concentration gradients, thus mimicking physiologic presentation. The principal investigator (PI) proposes to focus on differentiation of hESC toward the hematopoietic lineage because markers for intermediate stages along this differentiation path are well characterized. Moreover, both EB and monolayer differentiation can be used to generate hematopoietic cells, allowing comparison of differentiation strategies.

Reviewers felt that the proposed studies have a high potential impact on the stem cell field, as uniform, reproducible culture conditions are likely to facilitate better understanding of stem cell biology, and could significantly impact bioprocessing of stem cells and their differentiated progeny. The design and the approach are very interesting. Using microfluidics (hydrodynamic sorting), the PI proposes to control EB size, sort EBs by size, and thereby potentially control differentiation. The preliminary data illustrate the feasibility of the approach. On the other hand, many people have described ways to make uniform-size EBs including Zandstra (Stem Cells 2008, PLoS ONE 2008) Khademhosseini (Biomaterials 2008), Palacek (Biomaterials 2008), and Takayama (Lab Chip 2007). Reviewers thought the proposal would have been strengthened by a comparison of the proposed methods to those in the literature, especially addressing issues such as the ability of the hydrodynamic approach to handle non-spherical EBs, and the relative scalability of methods, and the novelty of details of the applicant's approach. The details of the sorting, as presented, are not sufficient to determine whether the approach represents a dramatic improvement over others. Reviewers commended the innovation employed in the proposal; they thought that the combination of engineering and stem cell biology is potentially powerful. However, a lack of clarity in some parts of the proposal was problematic. For example, the PI does not explain how the EBs will be grown and maintained in the device. Furthermore, the EBs will be embedded and immobilized in a hydrogel, but the effects of the gel on the diffusion of added factors is not adequately covered in detail. Additionally, the applicant does not clearly show how this system is scalable. Other reviewers felt that the technologies were not tied together well and that the applicant did not relate the technologic approaches to underlying biologic problems.

In Aim 2, the use of stencil patterning to create uniform colonies of hESCs was thought to be fairly mature with supportive mESC data provided. Reviewers thought that transitioning the technology to hESCs will pose some unacknowledged difficulty with regard to the smallest (50 um) colonies, given that the cell clusters seeded into the stencil openings are likely to be at least 50 um. Nonetheless, the larger sizes will almost certainly work. The last sub-aim looks at combining stencil patterning with microfluidics to expose patterned colonies to gradients. This aim will likely be more challenging than anticipated by the applicants. For instance, maintaining a stable gradient for the duration of differentiation (~days to weeks) will be extremely challenging, as the systems tend to drift over time without some sort of feedback control (Leduc, Lab Chip 2007). These engineering challenges were considered likely to be overcome, but reviewers expected to see more acknowledgment of challenges in the application.

The PI is a distinguished engineer and brings unique expertise to bear on problems in the field of hESC research. Though the PI lacks extensive stem cell biology background, members of the team are well versed in stem cell biology, giving the team as a whole the necessary expertise to execute the work.

The following Working Group members had a conflict of interest with this application: